Measuring Bioenergetic Profiles of Human Adipocytes

Measuring White Adipose Tissue (WAT) Mitochondrial Function Reveals Impaired Fatty Acid Stimulated Uncoupling From Obese Individuals

Application Brief

Introduction

White adipose tissue (WAT) is increasingly being recognized for its importance in regulating metabolic homeostasis, in addition to passive lipid storage. However, mature adipocytes and WAT are challenging to study because they are laden with lipid, and float on top of media. Consequently, the ability to culture differentiated, mature adipocytes and WAT on culture plates to measure mitochondrial respiration and glycolysis, and to generate a bioenergetic profile is attractive.

This Application Brief describes how to characterize mitochondrial function in adipose tissue from adherent adipocytes or tissue pieces using an Agilent Seahorse XF24 Analyzer. The results show that elevated cAMP levels increase lipolysis, which in turn increases uncoupling, and energy expenditure (EE) in WAT. This Application Brief also reveals a surprising and unexpected role for the mitochondrial permeability transition pore (PTP) in this uncoupling response.

Einav Shnaidman; *et al.*[1] first studied mitochondrial function in human adipocytes derived from preadipocytes, differentiated on Agilent Seahorse XF Cell Culture Microplates. The adipocytes were stimulated with isoproterenol (ISO), forskolin (FSK), or dibutyryl-cAMP (DB), agents that lead to increased cAMP signaling, and were then subjected to the mitochondrial stress test. The mitochondrial stress test measures basal respiration, proton leak (with the addition of oligomycin), and spare respiratory capacity (with the addition of an uncoupling agent). These analyses are possible using the Agilent Seahorse XF Analyzer, since the adipocytes do not need to be detached from the plates prior to the analysis.

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Figure 1 shows that basal respiration as measured by oxygen consumption (OCR) increases with increased cAMP levels. Application of the mitochondrial stress test demonstrated that the increased mitochondrial leak was due to increased lipolysis, and increased fatty acid (FA) levels. This increase in OCR can be blocked by scavenging the fatty acids using BSA, or by knocking down ATGL using siRNA, inhibiting lipolysis. Further studies using a variety of methods and compounds, including the application of siRNA in mature adipocytes, combined with XF measurements, showed that increased respiration appears to require the activation of Bax, which in turn regulates the opening of the PTP[1].

The uptake in increased respiration after increasing cAMP levels was also repeated in mouse gonadal adipose tissue, establishing that connective fat tissue can also be analyzed in the Agilent Seahorse XF Analyzer (Figure 1B). The response to ISO was also compared between adipocytes from lean and obese donors, and it was found that the response to ISO was impaired in adipocytes from obese individuals (Figure 2).

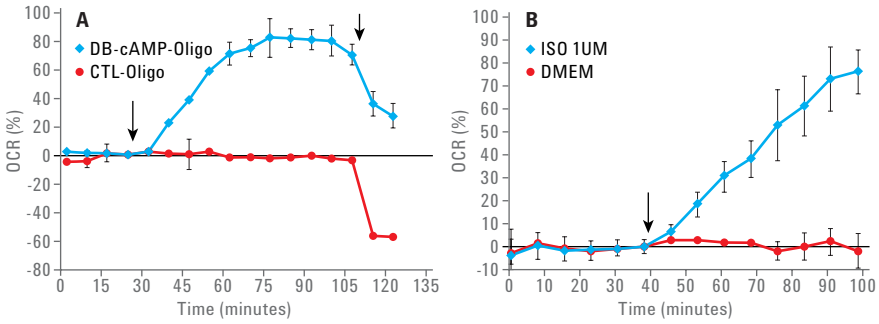

Figure 1. Increased cAMP in adipocytes or WAT leads to increased respiration. A) Respiration of adipocytes rapidly increases when treated with dibutyrylcAMP (DB). Oligomycin treatment reduces OCR in untreated control cells significantly more than in DB-cAMP treated adipocytes, suggesting increased leak (uncoupling) with increased cAMP levels. B) Mouse gonadal white adipose tissue (10 mg) pieces analyzed using Agilent Seahorse XF Islet Capture Microplates. An increase in cAMP is evoked by isoproterenol (ISO).

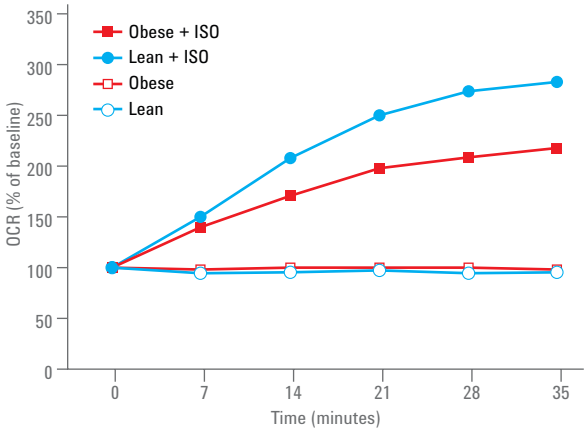

Figure 2. Respiration from human adipocytes derived from either lean (circles) or obese (squares) individuals. There is a marked decrease in the response to isoproterenol (open symbols) in adipocytes derived from obese people.

Results and Discussion

Increasing energy expenditure (EE) in WAT may be a method to combat obesity. Since the WAT in obese individuals occupies a substantial share of body mass, targeting this tissue is appealing. Gromada; *et al.*[2] treated human adipocytes with Fibroblast Growth Factor 21 (FGF21). The cells were then analyzed using the Agilent Seahorse XF24 Analyzer, and the mitochondrial stress test. It was found that treatment with FGF21 increased basal respiration, and more significantly, increased the cells capacity for oxidative metabolism.

In recent years, it has been revealed that adult humans have small deposits of brown adipose tissue (BAT) scattered among the WAT. This tissue expresses Uncoupling Protein 1 (UCP1) that uncouples ATP production from the oxidative phosphorylation by allowing H^+ to flow across the inner mitochondrial membrane producing heat. Thus, BAT is also a good model system for energy expenditure studies.

Johan Auwerx; *et al.*[3,4] have focused on the role of bile acids and their ability to increase EE in BAT. Using the Agilent Seahorse XF Analyzer, they showed that bile acids and mimetics increase EE via G-protein coupled receptor TGR5 and type II deiodinase. In another study, Auwerx; *et al.* examined EE in BAT using SIRT1 mimics. They previously showed that resveratrol, a constituent of red wine found in the skin of red grapes, can increase OCR, and thus EE, via SIRT1, and presented new compounds that can mimic this in BAT[5].

Hall; *et al.*[6] explored the role of type II deiodinase (D2), and thyroid hormones in the development of brown fat. Using the Agilent Seahorse XF Analyzer, they showed that brown fat adipocytes derived from D2 knockouts failed to increase OCR after stimulation, compared to wild type adipocytes.

Two studies deal with dysfunctional lipid metabolism in BAT and EE. Vergnes; *et al.*[7] used both adipocytes and BAT pieces to demonstrate that fatty acid binding protein 3 (FABP3) is necessary for efficient fatty acid (FA) oxidation in BAT, and that this alters cold tolerance. Ellis; *et al.*[8] used isolated mitochondria from BAT to show that long-chain acyl-CoA synthetase-1 (ACSL1) is also necessary for FA oxidation. The results obtained mimiced those from studies using radio-labeled FAs for FA oxidation.

Materials and Methods

Tissue

Freshly isolated mouse gonadal WAT was rinsed with Agilent Seahorse XF-DMEM containing 25 mM HEPES, cleaned of non-adipose material, and cut into pieces (~10 mg). After extensive washing, one piece of tissue was placed in each well of an Agilent Seahorse XF24 Islet Capture Microplate, and covered with the islet capture screen that allows free perfusion while minimizing tissue movement. Agilent Seahorse XF Assay Medium (500 µL) was added, and samples were analyzed in the Agilent Seahorse XF24 Analyzer.

Cells

Human pre-adipocytes (Zen-Bio, RTP, NC) were seeded into 0.2 % gelatin-covered 24-well Agilent Seahorse XF24 Cell Culture Microplates (13,000 cells/well) and grown for 24–48 hours in preadipocyte medium (PM-1, Zen-Bio) containing DMEM/F12 (1:1, v/v), HEPES (pH 7.4), 10 % FBS, and antibiotics. Cells were differentiated for seven days in medium containing DMEM/F12 (1:1; v/v), HEPES (pH 7.4), 10 % FBS, biotin, pantothenate, insulin, dexamethasone, IBMX, and a non-TZD PPARγ agonist (DM-2, Zen-Bio), followed by an additional week in adipocyte maintenance medium (AM-1, Zen-Bio: DM-2 without IBMX, and PPARγ agonist). The differentiated adipocytes were washed with 1 mL of XF-DMEM containing 1 mM sodium pyruvate, 2 mM GlutaMAX-1TM, 17.5 mM glucose, 1.85 g/L NaCl, 15 mg/L phenol red, pH 7.4), and 500 µL were added per well for assays.

XF Bioenergetic analysis

Bioenergetic analyses of white adipose tissue were performed in the Agilent Seahorse XF Analyzer. The Agilent Seahorse XF Analyzer creates a transient micro-chamber of only a few microliters in specialized cell culture microplates. This enables OCR and extracellular acidification rate (ECAR) to be monitored in real time.

Drugs were delivered to final concentrations of: ISO (1 µM), FSK (10 µM), oligomycin (Oligo, 1 µg/mL), FCCP (0.6 µM), and rotenone (3 µM). Optimal drug concentrations were determined in preliminary experiments. Basal respiration prior to drug administration is first measured followed by sequential addition of ISO or FSK, Oligo, FCCP, and rotenone. Rate measurements are taken between each addition. Rotenone will block mitochondrial respiration, thus any residual respiration is nonmitochondrial, and subtracted from the other rates. Respiration after Oligo will approximate leak (uncoupling) of the inner mitochondrial membrane and FCCP (titrated to give max respiration) allows for determination of respiratory capacity.

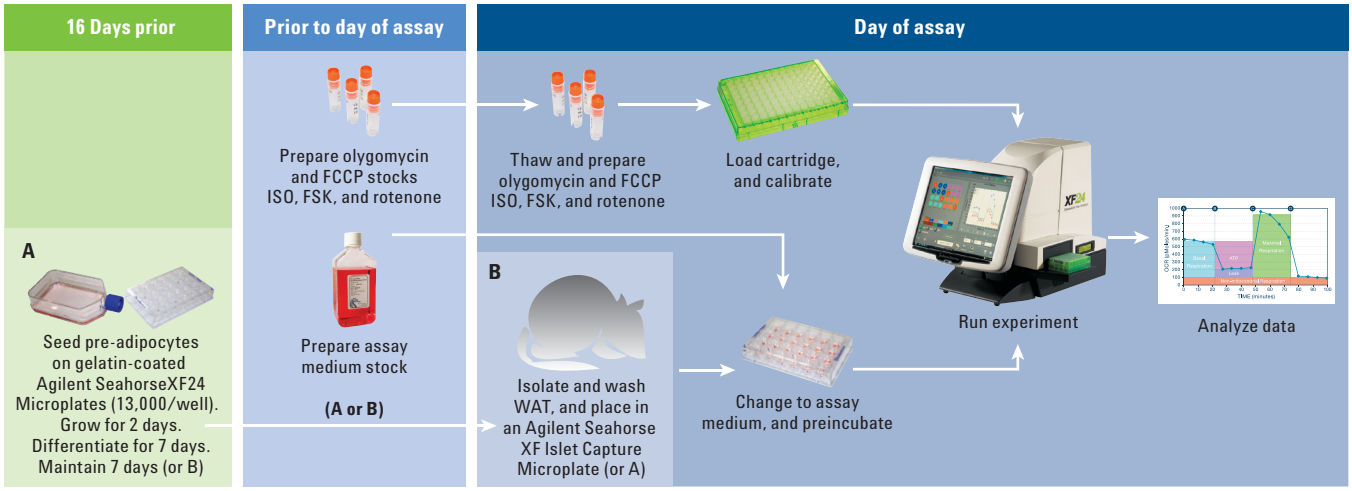

Figure 3. Flow chart of the XF assay.

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